

Supplementary Information

**Identification of novel and robust internal control genes from  
*Volvariella volvacea* that are suitable for RT-qPCR in  
filamentous fungi**

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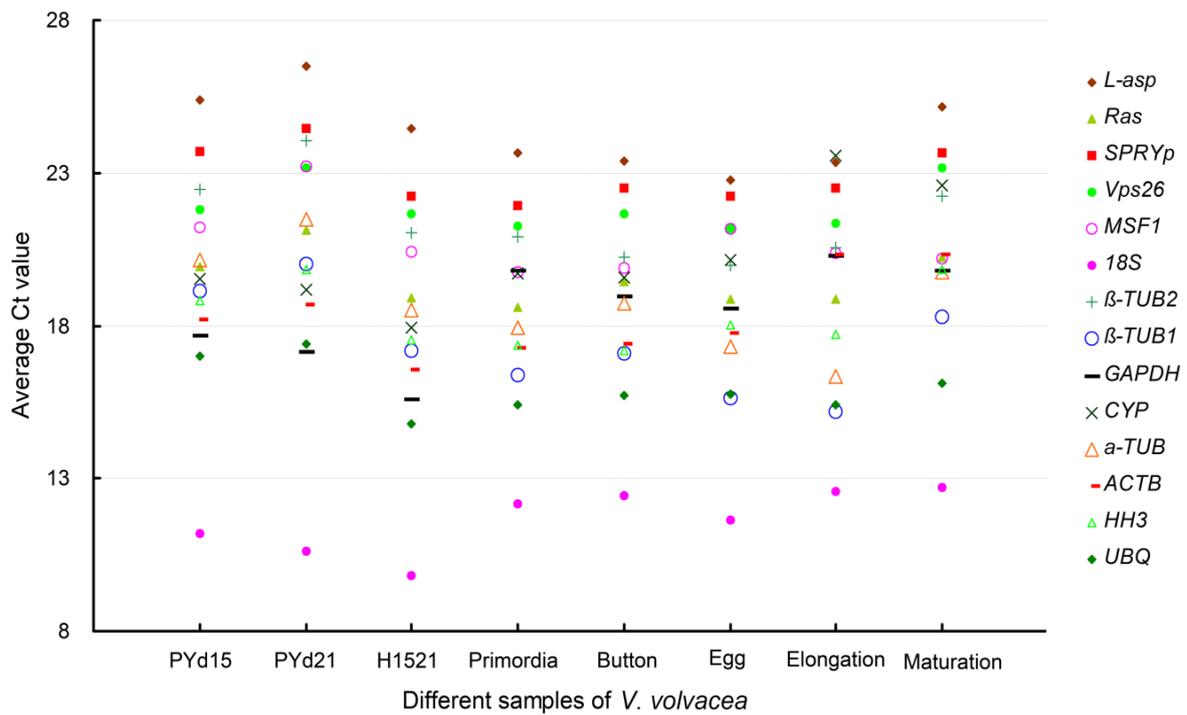
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**Figure S1.** RT-qPCR Ct values of fourteen ICGs in eight samples of *V. volvacea*

Mean Ct values of three biological replicates and three technical replicates are shown on the Y-axis. Different samples are shown on the X-axis: PYd15, homokaryon PYd15 mycelium; PYd21, homokaryon PYd21 mycelium; H1521, heterokaryon H1521 mycelium; primordia, button stage, egg stage, elongation stage and maturation stage are five developmental stages of fruiting bodies of *V. volvacea* strain H1521.

**Table S1.** The expression levels (TPM values, from digital gene expression profiles) of eight traditional housekeeping genes among eight expression profiles of *Volvariella volvacea*

Gene	PYd15 (TPM)	PYd21 (TPM)	H1521 (TPM)	Primordia (TPM)	Button stage (TPM)	Egg stage (TPM)	Elongation stage (TPM)	Maturation stage (TPM)	LOG <sub>2</sub> (MAX/MIN)
<i>ACTB</i>	2016.04	1220.49	2285.6	1549.14	707.55	750.85	173.96	505.87	3.72
<i>CYP</i>	1562.26	1066.39	1533.38	694.61	235.2	199.88	20.26	156.84	6.27
<i>GAPDH</i>	3923.63	6511.45	4756.06	541.06	389.32	341.89	155.88	942.53	5.38
<i>HH3</i>	111.58	410.69	88.22	125.63	68.06	227.15	90.75	44.91	3.19
<i>α-TUB</i>	9.91	3.53	6.66	0.71	4.28	19.4	17.92	5.53	4.77
<i>β-TUB1</i>	12.69	7.56	8.94	3.89	24.23	34.96	41.19	4.52	3.40
<i>β-TUB2</i>	78.21	69.74	131.54	78.28	82.14	257.42	49.9	84.45	2.37
<i>UBQ</i>	38.06	129.56	29.11	61.32	47.93	52.69	50.73	59.48	2.15

**Table S2.** Eight samples of *Volvariella volvacea* involved in this study and the four different combinations of sample sets

No.	Materials	Notes
1	PYd15 mycelium	Homokaryon
2	PYd21 mycelium	Homokaryon
3	H1521 mycelium	Heterokaryon
4	Primordia	Fruiting body of strain H1521
5	Button stage	Fruiting body of strain H1521
6	Egg stage	Fruiting body of strain H1521
7	Elongation stage	Fruiting body of strain H1521
8	Maturation stage	Fruiting body of strain H1521

The four different combinations of sample sets:

A	Different strains set	Samples of 1, 2 and 3
B	Different fruiting body developmental stages set	Samples of 4, 5, 6, 7 and 8
C	Different growth stages set	Samples of 3, 4, 5, 6, 7 and 8
D	Total samples set	Samples of 1, 2, 3, 4, 5, 6, 7 and 8

**Table S3.** Standard deviation of Ct values of fourteen candidate internal control genes using

## BestKeeper program

	<i>L-asp</i>	<i>Ras</i>	<i>SPRYp</i>	<i>Vps26</i>	<i>MSF1</i>	<i>18S</i>	<i><math>\beta</math>-TUB2</i>	<i><math>\beta</math>-TUB1</i>	<i>GAPDH</i>	<i>CYP</i>	<i><math>\alpha</math>-TUB</i>	<i>ACTB</i>	<i>HH3</i>	<i>UBQ</i>
N	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Geo Mean [Ct]	24.31	19.48	22.88	21.89	20.75	11.61	21.40	17.30	18.42	20.21	18.72	18.27	18.26	15.93
Ar Mean [Ct]	24.34	19.50	22.90	21.90	20.78	11.65	21.44	17.37	18.48	20.28	18.78	18.31	18.29	15.95
Min [Ct]	22.76	18.61	21.93	21.18	19.73	9.83	19.98	15.20	15.59	17.95	16.34	16.56	17.20	14.79
Max [Ct]	26.49	21.13	24.44	23.16	23.22	12.72	24.06	20.01	20.30	23.57	21.50	20.33	19.85	17.41
Std dev [ $\pm$ Ct]	1.04	0.70	0.77	0.63	0.82	0.83	1.11	1.34	1.26	1.40	1.27	1.10	0.91	0.67
CV [% Ct]	4.27	3.61	3.38	2.86	3.97	7.16	5.19	7.70	6.83	6.91	6.77	6.00	4.98	4.19

N: number of samples; Geo Mean: geometric mean of Ct values; Ar Mean: arithmetic mean of Ct values; Std dev [ $\pm$ Ct]: the standard deviation of the Ct; CV (%Ct): the coefficient of variance expressed as a percentage of the Ct level. The seven genes (*L-asp*,  *$\beta$ -TUB1*,  *$\beta$ -TUB2*, *GAPDH*, *CYP*,  *$\alpha$ -TUB* and *ACTB*) with highest standard deviation values (SD>1) are highlighted with red and removed in the first step (SD higher than 1 means high variation in expression).

**Table S4.** Correlation analysis of optimal internal control gene pairs using BestKeeper analysis

vs.	<i>Ras</i>	<i>SPRYp</i>	<i>Vps26</i>	<i>MSF1</i>	<i>18S</i>	<i>HH3</i>	<i>UBQ</i>
<i>SPRYp</i>	0.971	—	—	—	—	—	—
<i>P</i> value	0.001	—	—	—	—	—	—
<i>Vps26</i>	0.913	0.863	—	—	—	—	—
<i>P</i> value	0.002	0.006	—	—	—	—	—
<i>MSF1</i>	0.714	0.725	0.506	—	—	—	—
<i>P</i> value	0.047	0.042	0.200	—	—	—	—
<i>18S</i>	-0.189	-0.172	-0.094	-0.541	—	—	—
<i>P</i> value	0.655	0.682	0.826	0.165	—	—	—
<i>HH3</i>	0.877	0.914	0.892	0.644	-0.093	—	—
<i>P</i> value	0.004	0.002	0.003	0.085	0.826	—	—
<i>UBQ</i>	0.862	0.901	0.628	0.768	-0.109	0.778	—
<i>P</i> value	0.006	0.002	0.095	0.026	0.796	0.023	—

**Table S5.** The average absolute expression levels (TPM or RPKM values) of homologous genes of thirteen ICGs (except *18S*)

Internal control genes	Gene or protein ID	Mean TPM or RPKM
<i>Volvariella volvacea</i>	Gene ID	This study
<i>L-asp</i>	GME5360_g	6.30
<i>Ras</i>	GME11562_g	7.88
<i>SPRYp</i>	GME11387_g	61.76
<i>Vps26</i>	GME1490_g	89.46
<i>MSF1</i>	GME11630_g	156.28
$\beta$ - <i>TUB1</i>	GME2992_g	17.25
$\beta$ - <i>TUB2</i>	GME1291_g	103.96
<i>GAPDH</i>	GME5082_g	2195.23
<i>CYP</i>	GME7263_g	683.60
$\alpha$ - <i>TUB</i>	GME8406_g	8.49
<i>ACTB</i>	GME9359_g	1151.19
<i>HH3</i>	GME9377_g	145.87
<i>UBQ</i>	GME11487_g	58.61
<i>Flammulina velutipes</i>	ORF ID	Park <i>et al.</i> , 2014
<i>L-asp</i>	ctg11-2_AA_00476	3.62
<i>Ras</i>	ctg03_AA_00737	69.67
<i>SPRYp</i>	ctg03_AA_00882	29.53
<i>Vps26</i>	ctg11-1_AA_01092	11.83
<i>MSF1</i>	ctg03_AA_00662	168.11
$\beta$ - <i>TUB-1</i>	ctg03_AA_00282	76.87
$\beta$ - <i>TUB-2</i>	ctg01_AA_00278	536.67
<i>GAPDH</i>	ctg06_AA_00270	632.18
<i>CYP</i>	ctg05_AA_01318	1617.44
$\alpha$ - <i>TUB-1</i>	ctg05_AA_00452	134.12
$\alpha$ - <i>TUB-2</i>	ctg11-2_AA_00851	38.10
<i>ACTB</i>	ctg11-1_AA_00516	534.92
<i>HH3-1</i>	ctg13_AA_00215	254.72
<i>HH3-2</i>	ctg13_AA_00224	192.81
<i>UBQ</i>	ctg03_AA_00799	371.22

<i>Agaricus bisporus</i>	Protein ID	GSE65800	GSE39569
<i>L-asp</i>	63743	37.50	4427.50
<i>Ras</i>	133440	412.98	55015.00
<i>SPRYp</i>	60883	67.09	72.50
<i>Vps26</i>	194951	37.76	12820.50
<i>MSF1</i>	189859	217.21	2643.00
<i><math>\beta</math>-TUB-1</i>	195658	850.36	27686.25
<i><math>\beta</math>-TUB-2</i>	183821	208.21	8254.00
<i><math>\beta</math>-TUB-3</i>	187460	7.94	821.00
<i>GAPDH</i>	138631	1388.87	81562.75
<i>CYP</i>	138080	622.31	82610.50
<i><math>\alpha</math>-TUB</i>	135559	694.00	41241.75
<i>ACTB</i>	192120	899.53	51573.00
<i>HH3-1</i>	136889	125.63	5537.75
<i>HH3-2</i>	139997	772.18	18438.25
<i>HH3-3</i>	136959	189.30	28760.25
<i>UBQ</i>	133168	4393.59	101109.75

<i>Coprinopsis cinerea</i>	Gene ID	GSE58865
<i>L-asp</i>	CC1G_01149	658.59
<i>Ras</i>	CC1G_04430	1116.54
<i>SPRYp</i>	CC1G_06751	1849.61
<i>Vps26</i>	CC1G_09827	88.86
<i>MSF1</i>	CC1G_01701	532.76
<i><math>\beta</math>-TUB-1</i>	CC1G_04743	775.51
<i><math>\beta</math>-TUB-2</i>	CC1G_06184	67.91
<i>GAPDH</i>	CC1G_09116	4968.23
<i>CYP</i>	CC1G_09572	3299.42
<i><math>\alpha</math>-TUB-1</i>	CC1G_01375	190.76
<i><math>\alpha</math>-TUB-2</i>	CC1G_00146	611.51
<i>ACTB</i>	CC1G_08232	3460.98
<i>HH3-1</i>	CC1G_04396	9.67
<i>HH3-2</i>	CC1G_08799	4852.32
<i>HH3-3</i>	CC1G_05766	782.95
<i>UBQ-1</i>	CC1G_03676	115378.52
<i>UBQ-2</i>	CC1G_11833	4054.82

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<i>Laccaria bicolor</i>	Protein ID	GSE54789
<i>L-asp</i>	244034	4.64
<i>Ras-1</i>	291884	48.17
<i>Ras-2</i>	254501	1.49
<i>Ras-3</i>	256615	1.11
<i>SPRYp</i>	228899	0.96
<i>Vps26</i>	173065	70.81
<i>MSF1</i>	192412	254.62
<i>β-TUB-1</i>	294746	178.47
<i>β-TUB-2</i>	191405	32.93
<i>GAPDH-1</i>	295504	132.60
<i>GAPDH-2</i>	318873	335.16
<i>CYP-1</i>	294609	577.41
<i>CYP-2</i>	333157	3.86
<i>α-TUB-1</i>	192523	429.83
<i>α-TUB-2</i>	192524	69.92
<i>ACTB-1</i>	192701	532.52
<i>ACTB-2</i>	303826	9.77
<i>HH3-1</i>	170733	88.36
<i>HH3-2</i>	302849	52.13
<i>HH3-3</i>	294559	226.81
<i>HH3-4</i>	191988	90.97
<i>UBQ-1</i>	186716	1781.31
<i>UBQ-2</i>	192623	2716.81

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<i>Schizophyllum commune</i>	Protein ID	Ohm <i>et al.</i> , 2010
<i>L-asp</i>	66334	5.20
<i>Ras</i>	45883	74.07
<i>SPRYp</i>	64633	13.96
<i>Vps26</i>	77215	68.11
<i>MSF1</i>	73448	413.34
$\beta$ - <i>TUB-1</i>	77035	1113.84
$\beta$ - <i>TUB-2</i>	108932	164.41
<i>GAPDH</i>	78936	587.51
<i>CYP</i>	78835	1953.41
$\alpha$ - <i>TUB-1</i>	9815	286.28
$\alpha$ - <i>TUB-2</i>	72168	210.80
<i>ACTB</i>	83632	49.38
<i>HH3-1</i>	47315	28.07
<i>HH3-2</i>	67329	0.00
<i>HH3-3</i>	79839	0.00
<i>HH3-4</i>	27888	118.09
<i>HH3-5</i>	84991	97.27
<i>UBQ-1</i>	85500	2051.99
<i>UBQ-2</i>	64979	1475.51

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<i>Aspergillus nidulans</i>	Gene_Locus	GSE44100
<i>L-asp</i>	AN8169	40.73
<i>Ras</i>	AN5832	92.27
<i>SPRYp</i>	AN0831	81.20
<i>Vps26</i>	AN3642	41.33
<i>MSF1</i>	AN4295	259.37
$\beta$ - <i>TUB-1</i>	AN1182	287.63
$\beta$ - <i>TUB-2</i>	AN6838	131.54
<i>GAPDH</i>	AN8041	881.73
<i>CYP-1</i>	AN8605	3355.55
<i>CYP-2</i>	AN3814	392.60
$\alpha$ - <i>TUB-1</i>	AN0316	259.28
$\alpha$ - <i>TUB-2</i>	AN7570	220.80
<i>ACTB</i>	AN6542	663.66
<i>HH3</i>	AN0733	2515.32
<i>UBQ</i>	AN2000	2000.65

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<i>Fusarium graminearum</i>	Gene_Locus	GSE46133	GSE61865
<i>L-asp</i>	FGSG_07410	27.12	8.74
<i>Ras</i>	FGSG_10114	185.16	56.55
<i>SPRYp</i>	FGSG_01852	48.67	72.03
<i>Vps26</i>	FGSG_01155	65.55	66.35
<i>MSF1</i>	FGSG_10319	171.85	636.59
<i>β-TUB-1</i>	FGSG_09530	319.05	230.23
<i>β-TUB-2</i>	FGSG_06611	479.90	179.09
<i>GAPDH</i>	FGSG_06257	#N/A	2048.36
<i>CYP</i>	FGSG_00777	2278.81	806.81
<i>α-TUB-1</i>	FGSG_00639	502.80	239.65
<i>α-TUB-2</i>	FGSG_00397	635.39	208.75
<i>ACTB</i>	FGSG_07335	1395.45	849.19
<i>HH3</i>	FGSG_04290	3462.17	4262.53
<i>UBQ</i>	FGSG_08768	1581.34	1535.84

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<i>Trichoderma reesei</i>	Gene ID	GSE44648	GSE53629
<i>L-asp</i>	e_gw1.28.60.1	0.28	408.06
<i>Ras</i>	fgenesh5_pg.C_scaffold_23000098	5.05	1293.07
<i>SPRYp</i>	estExt_Genewise1.C_180178	8.92	3869.05
<i>Vps26</i>	e_gw1.12.211.1	2.20	1464.05
<i>MSF1</i>	estExt_GeneWisePlus.C_270244	20.98	3792.30
<i>β-TUB-1</i>	estExt_fgenesh5_pg.C_160102	24.57	3357.87
<i>β-TUB-2</i>	estExt_fgenesh1_pm.C_40092	20.78	4049.87
<i>GAPDH</i>	estExt_fgenesh5_pg.C_10410	166.09	43090.21
<i>CYP</i>	estExt_fgenesh5_pg.C_100037	63.22	6086.60
<i>α-TUB</i>	estExt_fgenesh5_pg.C_40423	9.10	5274.46
<i>ACTB</i>	estExt_Genewise1.C_11839	80.60	19940.54
<i>HH3</i>	estExt_fgenesh5_pg.C_390017	49.34	17729.02
<i>UBQ</i>	estExt_fgenesh5_pg.C_140156	130.28	45349.08

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<i>Neurospora crassa</i>														
	Protein ID	GSE36719	GSE42692	GSE35227	GSE45406	GSE53013	GSE60986	GSE44100	GSE53534	GSE44673	GSE60004	GSE52316	GSE51091	
	<i>L-asp</i>	NCU03768	72.89	46.50	27.66	59.99	35.67	11.03	10.56	20.47	7.61	2.35	4.10	21.34
	<i>Ras</i>	NCU03616	279.27	341.45	358.71	99.95	47.22	40.57	47.63	15.47	62.38	53.84	39.07	72.48
	<i>SPRYp</i>	NCU03678	418.47	318.04	358.76	51.40	18.07	19.36	61.92	22.01	68.41	57.18	85.49	36.61
	<i>Vps26</i>	NCU02743	332.95	354.06	459.59	101.42	41.42	34.80	55.00	16.06	64.29	33.94	43.78	39.99
	<i>MSF1</i>	NCU04307	321.26	238.71	140.23	100.92	19.24	18.59	19.80	7.81	42.22	13.45	83.47	39.85
	$\beta$ - <i>TUB-1</i>	NCU04054	4244.25	4298.39	4393.86	1032.81	781.86	679.69	572.29	357.00	603.88	244.14	498.89	675.09
	$\beta$ - <i>TUB-2</i>	NCU04054	4244.25	4298.39	4393.86	1032.81	781.86	679.69	572.29	357.00	603.88	244.14	498.89	675.09
	<i>GAPDH</i>	NCU01528	39886.06	28916.09	35633.73	3596.83	4027.27	2471.88	4029.20	16553.05	3931.17	1367.84	2725.67	3795.69
	<i>CYP</i>	NCU00726	17626.09	14551.15	19694.72	3740.68	3455.26	2060.09	2699.91	2017.39	2639.27	1315.66	2596.72	1782.67
	$\alpha$ - <i>TUB-1</i>	NCU09468	2144.33	2157.97	2595.15	524.84	541.01	286.44	330.92	203.35	269.16	127.40	261.33	306.31
	$\alpha$ - <i>TUB-2</i>	NCU09132	2721.44	2690.19	2649.47	778.30	435.10	336.48	312.43	174.84	317.88	138.15	229.98	340.93
	<i>ACTB</i>	NCU04173	6631.77	5579.17	3537.84	2007.65	1354.89	1174.95	1361.49	591.89	907.73	528.72	1040.19	1068.38
	<i>HH3</i>	NCU01635	15822.65	15619.89	17496.40	6499.16	2385.95	2112.48	2459.12	1389.77	3300.80	2239.55	1530.28	2393.35
	<i>UBQ</i>	NCU05995	6903.87	4328.64	7529.56	1366.28	488.72	308.99	768.61	305.97	1053.46	372.59	1146.08	423.30
Internal control genes	Gene or protein ID	Mean TPM or RPKM												